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Aquatic versus Mammalian Toxicology: Applications of the Comparative Approach

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The large body of literature and techniques generated by mammalian toxicity studies provides a conceptual and technical framework within which the absorption, fate, and disposition of xenobiotics in aquatic organisms can be studied. This review emphasizes the similarities and differences between mammalian and aquatic systems, e.g., lung vs. gill as site of absorption and toxicity. These must be taken into consideration when designing aquatic toxicity studies. Studies of phenol red in dogfish shark as an example show physiologic-based pharmacokinetic modeling to be a useful tool for investigating and eventually predicting species differences in xenobiotic disposition and drug differences within the same species. This discussion demonstrates that both laboratory and modeling procedures are now available to carry out sophisticated studies of xenobiotic fate and disposition in fish. Such studies are needed to pinpoint sites and mechanisms of pollutant toxicity in aquatic organisms.

Introduction

Reviewed here are the basic tenets of a pharmacokinetic assessment of xenobiotic toxicity. In so doing I hope to stress the interactions between body compartments and to provide examples that will assist the readers to integrate the more specific discussions which follow into an overall picture of the current state of our knowledge of pollutant action in aquatic organisms. In particular, the impact of specialized systems will be addressed, e.g., gills vs. lungs, on the fate, distribution, and toxicity of xenobiotics. Such considerations are very important if we are to avoid such fundamental flaws in experimental design as improper exposure route or conditions. Secondly, it will be shown that aquatic toxicology does not exist in a vacuum. Available for use are a wealth of experience and numerous sophisticated modeling devices developed in mammalian toxicological research, particularly in pharmacokinetic modeling. We need not rediscover the wheel. Finally, how some of the specializations of aquatic organisms may be used to advantage in assessing mechanisms of pollutant action in aquatic organisms and in all organisms will be described.

The Comparative Approach

Toxicology is the study of the harmful actions of substances on biologic tissues. Mammalian or warm-blooded terrestrial species are more commonly studied when one is concerned primarily with predicting health effects in humans. Aquatic and wild life species are studied when there is focus on potential environmental effects. In contrast, the comparative approach moves back and forth among species, but invariably is concerned with common fundamental biologic processes.

The basic tools and kinds of end points sought are similar in aquatic and classical toxicology. Aquatic toxicology is concerned with qualitative and quantitative aspects of the undesirable effects of xenobiotics on aquatic organisms. These undesirable effects range from the sublethal to the lethal and include changes in behavior, growth, development, reproduction, and pharmacologic responses, as well as underlying cellular effects at the histopathologic, biochemical, and physiologic levels. The field is also concerned with the concentrations of xenobiotics expected to occur in water, sediment, and food. This encompasses the transport, distribution, transformation, and ultimate fate of chemicals in our aquatic environment (*1*).

I see more similarities than differences between classical mammalian toxicology and aquatic toxicology. These similarities show up in a number of areas (Fig. 1). Those familiar with the mammalian field will recognize most of this material. This figure shows the starting point for mammalian mechanistic studies about 20 years ago. This is also where we are now in the aquatic area, since we have just begun or have yet to begin work on mechanisms in many of these areas.

Overview of Absorption, Fate, and Disposition of a Xenobiotic

Before exerting any pharmacologic or toxicologic effect, a xenobiotic must arrive at its site of action at a suitable concentration and remain there for an adequate

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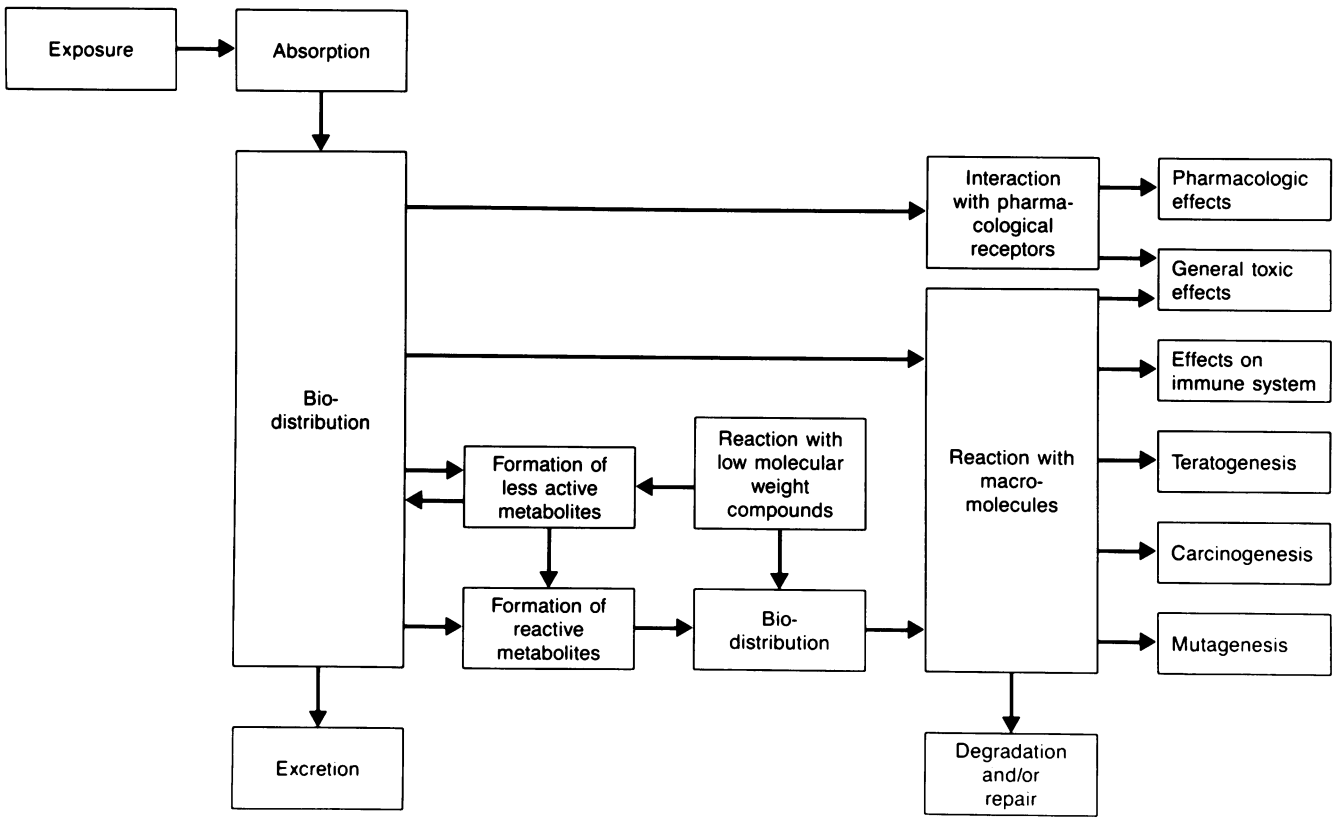

FIGURE 1. Overview of absorption fate and disposition of xenobiotics in mammals and fish.

length of time. Whether or not sensitive sites will be exposed to xenobiotic concentrations sufficient to impair function is determined by many processes, including absorption, biodistribution, excretion, metabolism, interactions with pharmacological receptors, and binding to macromolecules. The rate of onset of a particular action following xenobiotic treatment is influenced by the routes of exposure, mode of administration, and the rates of penetration through a succession of membranes. The duration of xenobiotic action is altered depending on rates of accumulation into tissues, metabolism, and excretion. Initially, species differences in xenobiotic activity were explained by qualitative and quantitative differences in the biotransformation of the compound. More recently, it has become apparent that there are also differences between species in the absorption, distribution, and excretion of chemicals (*2*).

The right side of Figure 1 depicts the six kinds of effects which can be demonstrated after exposure of an animal to a bioactive substance. All six kinds of effects have been invariably demonstrated first in terrestrial animals and later in aquatic species. Not shown are local effects which tend to be due to direct actions on the surfaces exposed to substances in the ambient media, e.g., fish skin and gill lesions from acid wastes and mammalian respiratory tract changes from exposure to gaseous toxicants. The "local" mechanisms, while certainly important, tend to be less interesting scientifically than systemic toxic effects.

In summary, Figure 1 shows the specific processes to be considered before undertaking studies of toxicologic mechanisms involving aquatic species. Many of these will be addressed in greater detail in these proceedings. Each process will be discussed here from three general points of view: (1) What do we know about its operation in aquatic species? (2) What have we learned from mammalian studies? (3) Can any of this knowledge be applied to aquatic species? Answers to these three questions clearly underlie the use of the comparative approach.

Exposure and Absorption

In most mammalian laboratory studies, xenobiotic exposure is by oral, intraperitoneal, or pulmonary routes. In the majority of fish studies, exposure is by addition of the test substance to the water. In the latter case, it may be difficult to dissect out the relative importance of oral, skin, and gill uptake. Thus, the block labeled "exposure" in Figure 1 requires some comparisons to be made between the two fields of toxicology, mammalian and aquatic.

The major fraction of absorbed xenobiotics comes from the ambient media from which terrestrial and

aquatic species obtain oxygen (air or water) and food. For example, during exchange of oxygen for carbon dioxide, chemicals in the surrounding air or water are brought to the surfaces of either of the two highly vascular organs, the lungs and the gills. At the air–lung or water–gill interfaces, physiocochemical factors determine the degree of xenobiotic absorption. These factors are the same ones that would determine rates of absorption by any route.

The physiocochemical factors that favor absorption of chemicals across mammalian membranes are well known. Most foreign compounds cross biologic membranes by passive diffusion and usually only lipid soluble and uncharged xenobiotics of low molecular weight readily diffuse through membranes (*3*). The degree of ionization is controlled by the pK_a of the compound and the pH of the body compartments in which the chemical is distributed. Many mammals have a gastric pH of about 1, an intestinal lumen pH of 5.3 to 7.8, and a plasma pH of 7.4. Thus, given the pK_a of a compound, one can predict the site and degree of absorption. For example, a xenobiotic which is a weak acid will be largely in the un-ionized form in the stomach and therefore readily diffuse across the stomach wall. Similarly, foreign compounds which are weak bases, and therefore partially un-ionized at the pH of the intestinal tract, will readily pass into the bloodstream from this site. In general, highly ionized molecules will not be absorbed from the gastrointestinal tract or gill unless a specific mechanism for transport exists. We know the stomach and intestinal tract pH for only a few aquatic species; thus, we are handicapped in predicting the degree of xenobiotic absorption.

It is often assumed that pharmacokinetic phenomena established in mammals also operate in fish. We recently had occasion to test this assumption in two species, channel catfish (*Ictalurus punctatus*) and striped bass (*Morone saxatilis*), under active oral treatment with oxytetracycline (OTC) (*4*). Very high gastrointestinal tract levels of the drug were found, but the liver had very low levels, and levels in muscle were negligible. In a follow-up study with ^{3}H-tetracycline, the bioavailability following oral administration was shown to be less than 5% (*4*). This was determined in the traditional way, i.e., by comparing the oral and intravenous area under the curve of drug concentration vs. time. Bioavailability was not substantially influenced by the presence of food.

Thus, an important gap in our comparative pharmacology has been discovered. There are no in-depth studies in any aquatic species that establish the optimal physiocochemical properties favoring gastrointestinal tract absorption. This is surprising, since many excellent nutritional studies have been conducted in aquatic species, and the techniques utilized for studying nutrient absorption generally could be applied to xenobiotics. One practical reason we need this information is that aquaculture is growing rapidly in the U.S. and, as seen in the intense farming of other animals, aquatic species succumb to bacterial diseases that can be treated with antibiotics. But which ones will work? Unfortunately, treatment has been hit-or-miss so far.

The above cited study is one of the first true bioavailability studies for a drug in fish. Recently, Varanasi and co-workers examined the bioavailability of the carcinogen benzo(a)pyrene, demonstrating its availability in contaminated sediments (*5*). Clearly, we need more fish bioavailability studies if we are to accurately assess both the therapeutic and the environmental impacts of drugs and other xenobiotics.

Having discussed absorption that occurs while ingesting food or while extracting oxygen from air or water, let us note a third exposure route—the skin. Here the barriers vary in structural complexity ranging from direct integument exposure to the imposition of extra barriers such as fur and scales or horns and hooves. Again, data quantifying dermal absorption of chemical entities in mammals are far more extensive than for aquatic species. While technically challenging, it is critical that studies which separately quantify rates of xeobiotic absorption from the water via the gills, gut, and skin be performed.

Biodistribution

Exposure conditions and absorption rates determine how xenobiotics get into the body. We must also consider where and how chemicals are distributed once they have entered the bloodstream, i.e., biodistribution. Some factors determining xenobiotic biodistribution are blood flow, plasma binding and transport, blood–organ barriers, tissue binding, and tissue uptake, both diffusion rate-limited and perfusion rate-limited.

Obviously, the rate at which blood flows through an organ is a prime determinant of how much of a chemical in the blood will reach the tissue. One major determinant of tissue perfusion is heart rate. One of the highest rates among mammals occurs in the mouse (600 beats/min). Fish such as eel and dogfish sharks have rates only one-tenth those seen in mice. It is important to note that physiologic-based pharmacokinetic modeling techniques can compensate for these wide differences and therefore provide a powerful tool for comparative studies.

Binding to plasma proteins is a second important process that determines the biodistribution of chemicals. In one sector, industrial drug development, plasma protein binding is routinely measured for new drugs. Only rarely does one see such studies where drugs or industrial contaminants are tested in fish. Since the component proteins in aquatic and mammalian plasma can be quite different, it is likely that the degree of protein binding may also be very different. Binding can alter the efficiency of tissue extraction of the chemical as the blood perfuses a given organ because of competition for xenobiotics between plasma proteins and tissue components. Compounds highly bound to plasma proteins relative to tissue sites will show higher retention in

plasma. Substances with high affinities for certain tissue components, e.g., heavy metals for sulfhydryl proteins such as metallothionein (*6*) and DDT for body fat, will show high tissue/plasma ratios. An overview of species differences in plasma protein levels and protein fractions (*7*) appears in Table 1.

Two points are particularly noteworthy: (1) the range of total protein levels is 1.7 to 11.0 g/100 mL; and (2) a number of protein fractions are undetectable (0.0%) in several species. Obviously these two factors will alter the qualitative and quantitative aspects of xenobiotic binding to plasma of different species.

Even though a blood-borne toxicant is presented to a certain organ, a blood-organ barrier may prevent its entry, thus protecting that organ from adverse effects. Perhaps the most famous of these is the blood–brain barrier. Some feel this is more a functional characteristic than a specific anatomic structure. Certainly, some exclusion of blood-borne chemicals has been shown by the brains of nearly every animal tested, even in those lower species without the complex mammalian type of blood-brain barrier (*8*). However, because of their simpler barriers, fish would be expected to offer less resistance to penetration of lipid soluble CNS intoxicants than most mammalian species. As a class, aquatic species do seem to be more sensitive than mammals to such CNS active chemicals.

The last factor determining biodistribution is tissue uptake. The rate of xenobiotic entry into tissue may be limited by diffusion barriers or by vascular perfusion rates. Uptake will be diffusion rate-limited if a substance's movement across membranes is slow. As a general rule, highly water-soluble compounds tend to be diffusion rate-limited. On the other hand, molecules are perfusion rate-limited if the rate of diffusion across membranes is rapid. Then tissue uptake is limited by the rate at which the chemical is delivered to the tissues, i.e., by the vascular perfusion rate.

Excretion

Toxicity of a given agent is not determined solely by its ability to reach a sensitive target site. The compound must also reach a sufficient concentration at the site to exert an effect. Therefore, the efficacy of excretion may profoundly influence the extent to which toxicity is expressed. Aquatic and terrestrial species utilize three primary routes of excretion (Table 2). In both classes of animals, two are similar, the urinary and biliary routes. Fish also excrete some substances via the gills, whereas mammals utilize the lungs as a way of ridding their body of certain chemicals. Other more specialized routes are also listed in Table 2.

Substances excreted in the urine of mammals are also

Table 1. Species differences in plasma levels of selected proteins.

Species	Total protein, g/100 mL	Protein in fractions, %				
		Albumin and prealbumin[b]	α 1-globulin	α 2-globulin	β globulin	γ globulin
Mammals						
Human	7.4	56.5	2.4	12.1	10.6	18.4
Monkey	8.8	56.0	2.7	6.6	29.0	5.6
Seal	4.9	53.3	3.6	20.8	15.8	6.5
Swine	11.0	46.5	0.0	20.1	16.6	16.8
Goat	6.3	63.8	8.1	0.0	15.6	12.5
Horse	6.9	41.3	0.0	14.7	12.7	31.3
Lamb	7.1	50.9	0.0	18.2	7.3	23.6
Cattle	7.8	41.8	0.0	14.4	12.9	30.9
Hamster	4.9	58.4	15.0	2.7	6.2	17.7
Guinea pig	3.6	59.1	24.0	4.2	2.8	9.9
Rat	6.7	47.1	27.0	0.0	18.4	7.5
Rabbit	5.7	67.9	2.2	12.4	9.6	7.9
Dog	9.0	29.2	4.1	19.9	13.3	33.5
Mouse	6.2	52.7	20.2	0.0	20.9	6.2
Cat	6.8	25.4	2.6	21.2	10.9	39.9
Aquatic						
Lobster	2.0	79.8	0.0	17.4	0.0	2.8
Dogfish	2.8	41.8	0.0	53.5	0.0	4.7
Amphibian						
Turtle	4.1	10.5	0.0	26.4	27.2	35.9
Frog	2.7	71.2	0.0	17.3	11.5	0.0
Avian						
Turkey	4.6	46.2	0.0	12.5	14.4	26.9
Duck	3.5	51.8	3.8	5.1	15.2	24.1
Pigeon	2.3	36.8	0.0	13.1	35.8	14.3
Goose	1.7	58.6	0.0	41.4	0.0	0.0
Chicken	3.5	57.1	0.0	15.6	27.3	0.0

[a] Revised from Guarino et al. (*1*). Standard total protein assay and plasma paper electrophoretic methods were used.
[b] Method used did not differentiate between pre-albumin and albumin and therefore is reported as composite.

Table 2. Major excretory routes.

Aquatic species	Terresterial species
Urine	Urine
Bile	Bile
Gills	Lungs
Others	Others
Eggs	Milk
Skin/mucus	Sweat

excreted in fish urine via roughly similar mechanisms. In fact, the development of our current understanding of renal function demonstrates that the "success of the comparative approach... is dependent upon anatomical differences between species" (*8*). When researchers were trying to elucidate the roles of the tubule and the glomerulus in urine formation, they realized that the goose-fish (*Lophius americanus*) was aglomerular and the hagfish (*Myxine glutinosa*) was atubular. Work in these two species, especially the goosefish, greatly clarified the functional role of the different anatomic structures of the kidney. It also demonstrated that nephron segment function is highly conserved, i.e., morphologically similar segments do similar jobs in fish and mammals.

Biliary excretion has not been as extensively studied in fish as in mammals. Dr. John Lech and I (*9*) recently reviewed this topic and noted that each of us had independently concluded more than a dozen years ago that mammals and fish were concentrating xenobiotics in the bile and that this process could be exploited for environmental and regulatory monitoring purposes. We reported (*9*) that for 44 drugs and other xenobiotics in several species of fish, concentrations of chemicals in bile most often were considerably higher than those in plasma or ambient water, with 38 of 44 compounds (86%) yielding bile/plasma or bile/water ratios greater than unity. Indeed, for about half of these compounds or their metabolites, concentration ratios exceeded ten. The tremendous analytic advantage of sampling the biliary compartment of fish is obvious. In mammals, a species-dependent molecular weight threshold favoring biliary transport has been proposed (*10*), with a typical range of 275 to 375 daltons reported for the rat. Our dogfish shark data indicate a higher threshold, in the 400 to 500 dalton range. Further work on other aquatic species needs to be done.

Metabolism and Other Reactions with Biomolecules

The second major general mechanism preventing accumulation of toxic agents within the cell is conversion to less toxic metabolites, i.e., deactivation or, as we used to say, detoxification. However, some chemicals are metabolically activated by inducible enzymes to form reactive metabolites.

Metabolites in turn are further biodistributed, and some react with low molecular weight compounds, such as glucuronic acid, or amino acids, such as taurine, yielding less active but more polar conjugates. Unstable metabolites can go on to react with macromolecules. If not degraded or repaired (Fig. 1) the adducts can produce a host of effects ranging from alterations in the immune system to reproductive, carcinogenic, or mutagenic responses. Clearly this is a highly complex and important area. Several of the subsequent papers in this volume deal with these questions, both for organic pollutants and metals.

The other side of the coin with respect to reactions with macromolecular species within the cell is that each of the six classes of toxicity shown on the right side of Figure 1 reflects interactions between xenobiotics (or metabolites) and biologically important macromolecules ranging from nucleic acids and receptors to enzymes and transport proteins. These interactions and their toxicologic manifestations are the focus of many of the other reviews covered in this volume.

Comparative Aspects of One Xenobiotic in One Fish Species

For the rest of this paper I will use data on one xenobiotic, phenol red, to show the similarities between mammals and fish in their handling of foreign compounds. This work, recently reviewed (*11*), will be outlined here, because it clearly demonstrates the presence of physiologic/pharmacologic mechanisms in fish which are remarkably similar to those in mammals. Furthermore, these data show that techniques currently available for marine fish permit the kind of sophisticated pharmacokinetic analysis necessary to focus in on mechanisms of pollutant action.

The studies were conducted in the dogfish shark (*Squalus acanthias*), but the procedures developed and employed can be readily adapted to other fish. Our choice of the dogfish shark was based on the early studies of Rall and Zubrod (*12*). In 1962, they demonstrated that the disposition (distribution of drug between red blood cell, brain, and muscle) of the antimalarial drug quinine was similar in dogfish sharks to that reported in terrestrial mammals. Despite its promise, use of the shark as a model for drug disposition studies required the development of practical techniques for sampling important physiologic compartments, such as the urinary and biliary systems, and validation of pharmacokinetic parameters. Phenol red, a drug with well-known affinities for the renal compartment, was selected for evaluating the shark model and the efficacy of these new techniques.

The methods for collecting urine and bile (via biliary fistula) have been described (*11*). The shark proved hardy enough to survive these surgical manipulations and could be routinely maintained for at least one week following the facile intravenous (IV) injection of drug. All of the following tissues and fluids were sampled routinely: blood, urine, kidney, muscle, CSF, brain, liver, and bile.

To determine the disposition of phenol red in the

shark, we studied the transport properties of phenol red in both the renal and hepatic systems. To obviate absorption problems, the drug was injected into the tail vein. Phenol red was rapidly and biphasically cleared from the plasma compartment with an initial $t_{0.5}$ of about 1 hr and a second phase $t_{0.5}$ of 8 hr. As early as 10 min after injection, phenol red was detectable in kidney tissue. Hepatic levels took longer to peak (about 2 hr) than kidney levels and decayed with a half-time of about 10 hr. The glucuronide metabolite was not detected in plasma, kidney, or liver. Both phenol red and its glucuronide were found in urine within 30 min; they increased in concentration for the first 2 hr and then declined. The amount of conjugated drug appearing in the bile ranged from 15 to 30% of the total material present. Although obviously an important excretory route, renal tissue never contained more than 6% of the administered dose, and the renal percentage declined rapidly after 1 hr. After 10 min the hepatic compartment contained about 18% of the administered compound; a peak occurred at 2 hr, and this compartment continued to contain large amounts of phenol red for up to 12 hr. The total amounts of phenol red handled by the urinary and biliary systems over 48 hr were about 40% and 50%, respectively. In these compartments, most of the material was parent compound rather than metabolite.

To estimate the impact of enterohepatic circulation, biliary and urinary excretion of phenol red in normal and in biliary fistulized sharks were compared. The intact animal normally excreted 49% of the administered dose in 48 hr into gall bladder bile. Of this, 20% was excreted as the glucuronide conjugate. In 48 hr, 41% of the administered dose appeared in the urinary compartment of intact animals. When bile was collected via fistula, essentially the same fraction (47%), of the administered dose appeared in the bile; however, compared with the intact animal, about twice as much was the glucuronide conjugate (44% vs. 20%). These data suggest that the liver synthesizes the glucuronide and readily excretes it into the bile. In the animals with fistulae, a large proportion of the administered dose was diverted, preventing the reabsorption of the glucuronide (presumably after hydrolysis) via the gastrointestinal tract. The urinary excretion in surgically treated animals confirmed this latter point, since animals with fistulae excreted only 23% of the dose in urine in 48 hr compared with 41% in intact animals.

Consistent with the effective biliary and urinary excretion, phenol red was actively transported from plasma into renal and hepatic compartments. For example, 6 hr after dosing, the kidney/plasma value for free phenol red was 6.4 and the urine/plasma ratio was 30.4. Similarly, the liver/plasma value was 3.9 while the bile/plasma ratio was 64.3. When the dose was increased from 10 to 100 mg/kg, the level of phenol red in the plasma increased roughly proportionally, i.e., 12.8-fold (Table 3). Renal tissue content also increased approximately 10-fold. On the other hand, liver, urine, and biliary compartments increased far less than plasma, i.e., saturated.

It was also possible to characterize the transport process involved using inhibitors of specific pathways. In this case, phenol red was known to be excreted in mammals via the organic anion transport system. Probenecid, the classical inhibitor for this system, significantly reduced renal tissue, urine, and bile levels of free phenol red and its glucuronide (*11*). The effect was

Table 3. Effects of different doses of phenol red on its distribution in the dogfish shark.[a]

Tissue or fluid (form of phenol red)	Dose, μg/g or mL		
	10 mg/kg	100 mg/kg	Increase
Plasma (free)	16	204	12.8
Kidney (free)	83	948	11.4
Liver (free)	38	155	4.1
Urine (free)	711	1777	2.5
Urine (glucuronide)	63	148	2.3
Bile (free)	262	936	3.6
Bile (glucuronide)	120	241	2.0

[a] Animals were treated intravenously with 10 or 100 mg/kg phenol red. Values are means of five or six animals per dose 4 hr after treatment.

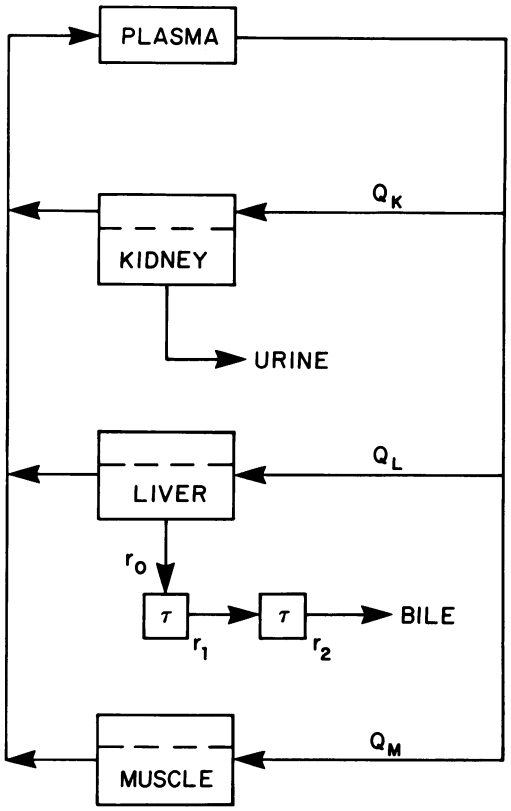

FIGURE 2. Body compartments important in phenol red distribution studies in dogfish shark. Q_i is the plasma flow rate in mL/min through kidney (K), liver (L), and muscle (M); r_i is the total rate of phenol red and glucuronide transported in terms of μg/min from the liver (i = 0) and bile duct (i = 1,2) compartments; and T is the nominal residence time for each bile duct compartment in minutes. From Bungay et al. (*13*).

somewhat more dramatic on the uptake of the glucuronide, suggesting that probenecid may be inhibiting both the transport and metabolism of phenol red. Probenecid pretreatment *in vivo* also caused a slight but significant decrease in the amount of phenol red binding in plasma.

Thus, study of the model compound, phenol red, enabled us to determine that the following features traditionally studied in mammalian systems could also be readily studied in fish: overall disposition and metabolism, effects of biliary fistula, effects of active transport *in vivo*, saturability of urinary and biliary excretion mechanism, inhibition of renal and hepatic transport by probenecid, and the presence of significant plasma protein binding. Furthermore, the data were qualitatively similar to that obtained in mammals.

Having developed these experimental techniques for the dogfish shark, we next established the applicability of pharmacokinetic scaling processes in the direction opposite that usually taken; pharmacokinetic models developed for mouse and extrapolated to man were used to go the other way, i.e., from mouse to fish (*13*). The general purpose of developing pharmacokinetic models is to describe the time course of xenobiotic disposition processes in the body. Thus, if it is possible to extrapolate back and forth between mammals and fish, it becomes possible to utilize fish where they provide experimental advantages and still make informative predictions of mammalian xenobiotic disposition.

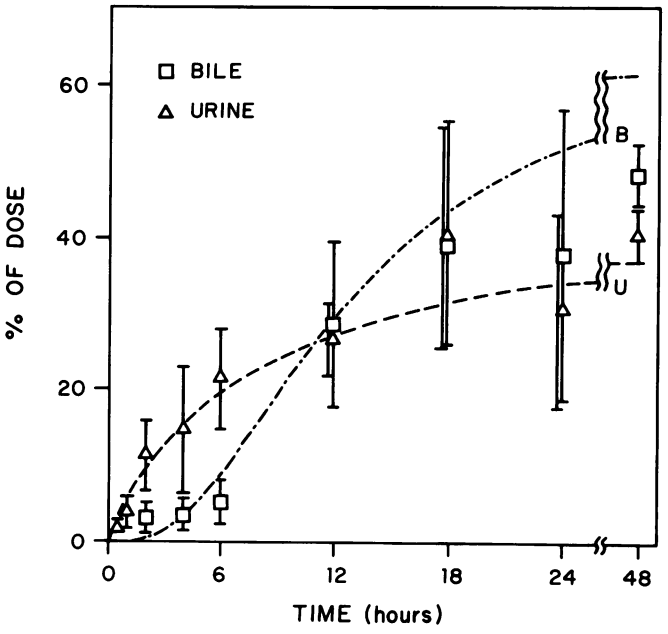

FIGURE 4. Time course of accumulation of phenol red and glucuronide in bile and urine. Lines are model predictions and symbols are experimental data: (□) bile (B); (△) urine (U). From Bungay et al. (*13*).

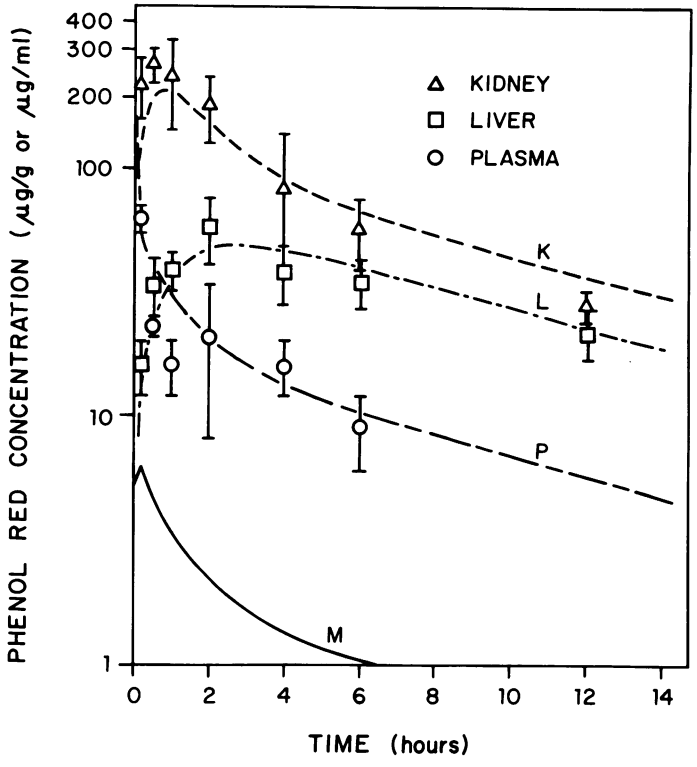

FIGURE 3. Model predictions vs. experimental results for time course of tissue and plasma concentrations of phenol red. Lines are model predictions; symbols are experimental data for IV injection of 10 mg/kg into caudal vein of dogfish sharks. Each symbol represents average of 5–8 female sharks/time point with SD indicated by vertical bars. Limit of sensitivity of assay was 25, 15, and 5 μg/g or mL for (△) kidney (K); (□) liver (L); and (○) plasma (P), respectively. From Bungay et al. (*13*).

In a typical physiological pharmacokinetic model (Fig. 2), physiological parameters such as the blood flow rate and blood volume of organs and tissues are used along with biochemical and physiocochemical parameters such as plasma protein binding, lipid solubility, and tissue/plasma concentration ratios (partition coefficients). The resulting model (Fig. 2) is composed of a series of compartments representing organs or tissues within which the xenobiotic concentrations are assumed to be uniform. One writes differential mass balance equations for each of these compartments to describe the inflow, outflow, accumulation, or disappearance of the xenobiotic. A key feature of the physiologic-based approach to pharmacokinetic modeling is that it can provide the means for predicting xenobiotic concentrations in tissues that may be the target of certain toxic actions. Furthermore, it is adaptable to changing physiologic circumstances, such as might occur in going from one species to another. In this model (Fig. 2), if the levels of a drug in the major organs at several time points are known, only the plasma flow for each compartment (Q values in mL/min) are needed.

Predictions obtained from the shark model are shown in Figure 3. Also shown in Figure 3 are the actual data points we collected for phenol red organ distribution (*13*). The urinary and biliary data are shown in Figure 4. Clearly, the collected data and the model predictions agree well (*13*).

In conclusion, even a practical regulatory agency, such as FDA, has acknowledged the need for a comparative species approach. Appearing in a 1983 Federal Register notice (*14*) was the following statement: "The ability to extrapolate reliably between species will depend on the amount of information available to the agency regarding the species involved, the nature of the disease or infection, the metabolism of the species involved, etc. The regulation contemplates a great deal of extrapolation between species." The dogfish shark has yielded excellent data consistent with similar studies on terrestrial mammals. The major transport and metabolic parameters in this fish were shown to be similar to those found in mammals. There is no reason why the techniques now established for this shark could not be applied with minor modifications to a number of other species and xenobiotics of interest to the aquatic toxicologist. Indeed, such studies of xenobiotic fate and distribution are critical to pinpoint sites and mechanisms of pollutant toxicity in aquatic organisms.

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